

Application No. 10/624,555
Reply to Office Action of April 9, 2003

REMARKS

Claim 1 is amended. Claim 10 is new. Support for the amendment is found at original Claims 8 and 9. Claims 1-8 and 10 are pending. Favorable consideration is respectfully requested.

At the Outset, Applicants thank Examiner Marvich for the helpful suggestions during the courteous discussion of the present invention which is summarized and expanded upon below. Further, Applicants thank Examiner Marvich for indicating that the above amendment and the comments below may further favorable prosecution of the present invention.

The rejection of Claims 1-9 under 35 U.S.C. § 112, first paragraph is believed to be obviated by the amendment above combined with the remarks below.

The Office contends that the specification, while being enabling for a method of producing a target substance utilizing E. coli with an enhanced SoxM type oxidase or NDH-I activity and deficient cytochrome bo type oxidase or NDH-II activity, it does not provide an enabling disclosure for a method using any microorganism with any enzyme constituting the respiratory chain pathway with high energy that is enhanced and/or with low energy efficiency that is deficient.

In support of the above contention, the Office takes the position that the specification, as well as the prior art, solely teaches that the cytochrome bd type oxidase or NDH-II are low efficiency enzymes while cytochrome bo type oxidase or NDH-I are high efficiency enzymes and that no other enzymes are exemplified. Moreover, the Office contends that neither the prior art nor the specification indicates that the means to identify and any other respiratory enzyme with low or high efficiency is available. Finally, the Office contends that it is not clear that applicant's invention can be performed with any microorganism with any enzyme

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constituting the respiratory chain pathway with high energy that is enhanced and/or low energy efficiency that is deficient.

Applicants respectfully disagree and respectfully submit that microorganisms other than *Escherichia* bacterium and *Coryneform* bacterium also have respiratory chain enzymes involved in a respiratory chain pathway. To support Applicants position, Applicants provide herewith six reference documents listed on the attached Information Disclosure Statement (D1-D6) that demonstrate a microorganism other than *Escherichia* bacterium and *Coryneform* bacterium also has respiratory chain enzymes involved in a respiratory chain pathway, where such enzymes are similar to those of *Escherichia coli*.

D1: J. Bacteriol., 180., 6571-6580.(1998)

D2: J. Biol. Chem., 267, 10225-10231 (1992)

D3: Biochem. Biophys. Acta, 1411, 147-158 (1999)

D4: Biophysical Journal, 76, 438-442 (1999)

D5: Microbiology, 147, 2865-2871 (2001)

D6: Arch. Microbiol., 173, 390-397 (2000)

D1-D4 disclose a microorganism having SoxM type oxidase, high energy efficiency enzyme, and cytochrome bd type oxidase, low energy efficiency enzyme, wherein the microorganism is a microorganism other than *Escherichia* bacterium and *Coryneform* bacterium. As such microorganisms, *Bacillus subtilis* (D1), *Paracoccus denitrificans* (D2), *Synechocystis* sp. (D3), *Pseudomonas aeruginosa* (D3), *Klebsiella pneumoniae* (D3), *Azotobacter vinelandii* (D3) and *Bacillus stearothermophilus* (D4) are exemplified (See abstract of D1, Fig. 4 of D2, Fig. 6 of D3 and abstract of D4).

D5 discloses a phylogenetic tree for subunit II of haem-copper oxidases which are a kind of SoxM type oxidase (See Fig. 5 of D5). This means that microorganisms in Fig. 5 of D5, for example, *Corynebacterium glutamicum*, *Mycobacterium tuberculosis*, *Bacillus*

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thermodenitrificans, and *Thermus thermophilus*, have SoxM type oxidases or similar family members thereof. Also Fig. 4 of D5 shows the similarity of SoxM type oxidases between microorganisms. D6 discloses multiple alignment of cytochrome bd sequences (See Fig. 7 of D6). Fig. 7 of D6 shows the similarity of cytochrome bd oxidases between microorganisms.

In view of the above-mentioned disclosures of D1-D6, it is clear that it was known by a person skilled in the art that a microorganism other than *Escherichia* bacterium and *Coryneform* bacterium have respiratory chain enzymes involved in a respiratory chain pathways when the instant application was filed. Further, these references demonstrate that it was known by a person skilled in the art that such enzymes are similar to those of *Escherichia coli*. Thus, a microorganism other than *Escherichia* bacterium and *Coryneform* bacterium also has two kinds of respiratory chain pathways comprising the respiratory chain pathway of high energy efficiency and the respiratory chain pathway of low energy efficiency as well as those of *Escherichia coli*.

In addition, due to the structural similarity of the enzymes between microorganisms (See Fig. 5 of D5 and Fig. 7 of D6), a person skilled in the art could isolate the enzymes from a microorganism, even when the microorganism is a microorganism other than *Escherichia* bacterium and *Coryneform* bacterium.

Means for enhancing activity of a respiratory chain enzyme of high energy efficiency, and means for reducing or eliminating activity of a respiratory chain enzyme of low energy efficiency are described in page 11, line 9 to page 19, line 8 of the present specification. Therefore, Applicants respectfully submit that, based on the description of the present specification, combined with the disclosures of D1-D6 demonstrating the level of skill in the art, a person skilled in the art can perform the present invention without undue experimentation.

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In light of the above, Applicants respectfully submit that such a high level of skill in the art combined with such a detailed disclosure directing a person how to obtains means for enhancing activity of a respiratory chain enzyme of high energy efficiency, and means for reducing or eliminating activity of a respiratory chain enzyme of low energy efficiency are described, clearly outweigh and overcome any unpredictability that may have been present long ago.

The Office relies on multiple disclosures to support a contention of high unpredictability in the art. Most of the disclosures are very old compared to the filing date of the present application. However, the Office has relied on Parekh published one year prior to applicants filing date; and therefore, merits discussion.

Applicants respectfully disagree on the basis that the Office has not considered Parekh as a whole or in its entirety. More specifically, Parekh which is relied heavily upon to support the Office's position further specifies that "This empirical approach has a long history of success," (see page 288, column 1, lines 28-31). Further, Parekh discloses that "Regardless of the methods or strategy, strain improvement relies on the iteration of three operations: genetic alteration, fermentation, and assay", demonstrating that there is no unpredictability in the art because one can easily iterate such operations that are well known. Then, Parekh proceeds to explain all of the tools available in the art to the skilled artisan in order to iterate the "three operations: genetic alteration, fermentation, and assay" on the ensuing 11 pages. In light of the full disclosure of Parekh, it is clear that the level of unpredictability is low when the skilled artisan is equipped with all of the tools and knowledge described by Parekh to iterate the "three operations: genetic alteration, fermentation, and assay" in order to achieve an improved strain.

Finally, the Office relies on Parekh to demonstrate that scale-up procedures can be troublesome. It should be noted that while Applicants do not eliminate the possibility of

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scaling up the claimed invention, Applicants do not understand how this impacts unpredictability in the claimed invention when the claims do not recite such an embodiment. Applicants respectfully submit that it is inappropriate for the Office to rely on disclosures related to scale-up procedures when such procedures are not recited in the claims.

In light of the above, Applicants respectfully submit that the Office has not met the standard necessary, nor provided the Applicants with adequate evidence, to maintain the rejection under 35 U.S.C. § 112, first paragraph. Accordingly, withdrawal of this ground of rejection is respectfully requested. If the Office maintains the rejection, the Applicants respectfully request that the Office issue a non-final Office Action in light of the above remarks.

Finally, Applicants respectfully request the Draftsman and the Examiner to consider the Figures attached herewith which were previously submitted with Applicants' last Response. Unfortunately, it appears as if the Figures filed with the last Response was not considered. Therefore, Applicants' provide herewith another copy of such Figures for the Office's convenience.

Applicants respectfully submit that the present application is now in condition for allowance. Should anything further be required to place this application in condition for allowance, the Examiner is requested to contact the Applicants' attorney by telephone.

Respectfully submitted,

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